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RESEARCH ARTICLE

Assessing phenotypic, biochemical, and molecular diversity in coriander (*Coriandrum sativum* L.) germplasm

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Abstract Our goals for this research were to elucidate phenotypic and biochemical diversity in coriander (*Coriandrum sativum* L.) populations maintained at the North Central Regional Plant Introduction Station in Ames, IA, and examine relationships between amplified fragment length polymorphism (AFLP) markers and patterns of phenotypic and biochemical diversity. Phenotypic and biochemical traits were evaluated, and analyses of variance and mean comparisons were performed on the resulting data sets. Euclidean distances from phenotypic (PD) and biochemical (BD) data were estimated, and modified Rogers' distances (RD) were estimated for 80 polymorphic AFLP markers. These data were subjected to cluster analyses (CA) and principal components analyses (PCA), to reveal patterns among populations, and to analyses of molecular variance (AMOVA) for grouping patterns from PD and BD by using the 80 polymorphic AFLP markers. Resulting phenotypic, biochemical, and molecular distance matrices were also compared by applying Mantel tests. Our results describe significant differences among populations for all the phenotypic traits, and dendrograms obtained from PD and BD revealed complex phenetic patterns, as did groups from PCA. The primary seed essential oils and nearly all fatty-acid components were identified and their abundance measured; the primary chemical

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constituents of corresponding PCA groups are described herein. Molecular evidence supported phenotypic and biochemical subgroups. However, variation attributed among subgroups and groups was very low (~4–6%), while variation among populations within groups was intermediate (~24–26%), and that within populations was large (~69–70%), reflecting weak differentiation among subgroups and groups, which was confirmed by values for fixation indices. Phenotypic subgroups described in this study differed somewhat from previous infraspecific classifications. Weak correlations were found between the phenotypic and biochemical matrices and between the biochemical and AFLP matrices. No correlation was found between the phenotypic and AFLP matrices. These results may be related to coriander's phenotypic plasticity, its wide range in lifecycle duration, its predominantly allogamous reproductive biology, a human-selection process focused on special traits that may be controlled by few genes, and the widespread trade of coriander seeds as a spice, which may result in dynamic, poorly differentiated molecular variation, even when phenotypic and biochemical differentiation is easily documented.

Keywords AFLP · Analysis of Molecular Variance · Biochemical Profile · *Coriandrum sativum* · Cluster Analysis · Gas Chromatography Analysis · Phenotypic Traits · Principal Components Analysis

Introduction

Coriander (*Coriandrum sativum* L.), is an annual herb that belongs to the umbel family (Apiaceae). It displays broad adaptation as a crop around the world, growing well under many different types of soil and weather conditions (Guenther 1952; Purseglove et al. 1981; Simon 1990), even at extreme latitudes and elevations. In addition, the short life cycle in most coriander cultivars allows farmers to fit their cultivation into some part of the growing season in almost any region. Coriander has long been cultivated in the Mediterranean region, southern Europe, Asia Minor and the Caucasus. In recent years, principal commercial coriander producers included members of the former Soviet Union, Hungary, Poland, Romania, Czech Republic, Slovakia, Morocco, Canada, India, Pakistan, Iran, Turkey, Guatemala, Mexico, and Argentina (Kiehn and Reimer 1992; Agri-facts 1998). The fresh green herb and a dry spice are the two main products obtained from coriander plants. The herb is a perishable product, generally grown for domestic consumption in Asia, the Middle East, Central and South America. The spice is the dried form of the whole, mature fruit and is used in whole or ground form as a flavoring; the spice is also employed for the preparation of steam-distilled essential oil and solvent-extracted oleoresin for the aroma and flavor industries (Purseglove et al. 1981; Kiehn and Reimer 1992; Agri-facts 1998). Coriander seed oil is included among the 20 major essential oils in the world market (Lawrence 1992), and its commercial value depends on its physical properties, chemical composition, and aroma (Smallfield et al. 2001). The essential-oil content of the dried fruits varies from very low (0.03%) to a maximum report of 2.7% (Purseglove et al. 1981; Bandara et al. 2000). Linalool is the main volatile compound in seeds, typically constituting more than 50% of the total essential oil. Fatty acids are also important components of coriander seeds; the main fatty acids detected in coriander, in decreasing order, are petroselenic, linoleic, palmitic, and stearic acids (Ramadan and Mörsel 2003). Furthermore, cleavage of the unusual double bond in petroselenic acid leads to the production of lauric acid, to obtain surfactants and edible products, and adipic acid for nylon synthesis (Kleiman and Spencer 1982; Isbell et al. 2006). Residues from distillation can be used for livestock feed, and the fatty acids also have potential uses as lubricants (Purseglove et al. 1981).

Purseglove et al. (1981) divided coriander by fruit size into two types. The large-fruited types (referred to as var. *vulgare* Alef. = var. *sativum*) are mainly produced in tropical and subtropical countries and produce seeds that contain about 0.1 to 0.35% volatile oil; these plants typically have a short lifecycle (Agri-facts 1998). The small-fruited types (var. *microcarpum* DC.) are more commonly produced in temperate regions and usually have seed with volatile-oil content greater than 0.4%. Ivanova and Stoletova (1990) described four geographic centers for different types of coriander: (i) India, (ii) Northern Africa, (iii) Central Asia, and (iv) Abyssinia and recognized four corresponding subspecies within *C. sativum* (*indicum* Stolet., *sativum*, *asiaticum* Stolet.,

and *vavilovii* Stolet.). Diederichsen (1996) conducted an extensive analysis of morphological variation, and on that basis proposed the following ecogeographic types: Near Eastern, Indian, Central Asian, Syrian, Caucasian and Ethiopian. The most recent study of infraspecific variation in coriander was carried out by Diederichsen and Hammer (2003), who examined variation patterns for phenological and morphological traits along with seed essential-oil and fatty-acid content (FAC). Those authors proposed three subspecies: *sativum*, *microcarpum*, and *indicum*, with 10 botanical varieties assigned among them.

Characterization of crop genetic diversity has long been based on morphological traits, which often have notable advantages, such as straightforward detection and measurement and relevance to characters of importance to germplasm users. However, they can also have serious limitations. Many are controlled by multiple alleles and loci, making it difficult to relate patterns of phenotypic variation to their genetic bases, and their expression may be strongly affected by the environment (Lombard et al. 2001; Nuel et al. 2001). Still, most investigations of variation within and among coriander populations have been based on morphological traits, although many of those traits are environmentally influenced, and genotype-by-environment interactions are common (Jindla et al. 1985; Sastri et al. 1989; Bhandari and Gupta 1991; Angelini et al. 1997; Ali et al. 1999).

Chemotaxonomy has been used to elucidate systematic relationships at various taxonomic levels, and, although hundreds of volatile compounds can be produced in aromatic plants, relatively few are typically responsible for characteristic aromas or flavors (Goff and Klee 2006) and even fewer are generally used to define chemotypes. Chemical profiles of volatile compounds are widely used to help establish systematic relationships among plant populations, but these secondary metabolites are most useful in taxonomic classification only when other factors, such as environmental conditions, plant development, and extraction methods, are carefully standardized (Vieira et al. 2001; Pichersky et al. 2006). In contrast to widespread usage of essential oils in plant systematics, fatty-acid profiles are somewhat less commonly applied to the study of infraspecific variation in plants. Seiler and Brothers (1999) detected wide variation in oil concentration and fatty-acid composition among six wild sunflower species (*Helianthus*). Searching for chemotaxonomic relationships among selected crucifer genera, Miller et al. (1965) reported uniformity in fatty-acid composition for certain genera but variability within others, even within species. By combining use of molecular markers and fatty-acid profiles to study white mustard (*Sinapis alba* L.) accessions, Granot et al. (1996) documented genetic variability among accessions, as well as among them and even within populations within accessions, and a relationship was found between genotypes and the content of erucic acid.

In contrast to morphological and biochemical traits, molecular markers based on DNA polymorphisms are generally not affected by environment. They are widespread within plant genomes, and techniques are improving to make them more reliable and efficient (Ramanatha and Hodgkin 2002). The choice of an appropriate molecular marker depends on many considerations, with no single approach optimal for studying infraspecific variation or for solving the needs of *ex situ* germplasm conservation (Spooner et al. 2005), such as the identification of duplicate accessions and gaps in genebank collections or the development of effective regeneration systems. Important classes of DNA-based molecular markers, which have been used for genetic-diversity assessment, cultivar fingerprinting, and phylogenetic studies, include AFLPs, as well as restriction fragment length polymorphisms (RFLP), random amplified polymorphic DNA (RAPD), and single sequence repeats (SSR) (Brown et al. 1996; Heslop-Harrison and Schwarzacher 1996; Penner 1996; Reiter 2001; de Vienne et al. 2003; Bhat et al. 2004). Briefly, AFLP is a PCR-based molecular-marker class (Vos et al. 1995), typically considered within the group of dominant sequence polymorphisms (de Vienne et al. 2003). AFLP markers combine advantages from RFLP markers and from the PCR technique (Vos et al. 1995), such as reliability and the capacity to increase exponentially the copy number of selected fragments, respectively (Hartl and Jones 2001). The AFLP technique seems to be more reliable than other PCR-based molecular markers (Reiter 2001). In addition, this technique has an option for high throughput by means of multiplexing primer pairs and automated fragment detection and scoring (Roldán-Ruiz et al. 2000; Bhat et al. 2004). Despite their utility for elucidating genetic relationships

within plant species, molecular markers have not yet been applied to questions of infraspecific classification or fingerprinting in coriander.

Two hundred and seventeen accessions of coriander from throughout the world are conserved at the North Central Regional Plant Introduction Station (NCRPIS) in Ames, IA. Our goals were to evaluate coriander accessions conserved at the NCRPIS, characterizing extant phenotypic and biochemical variation, and to test variation patterns by applying AFLP markers for analysis of molecular variance (AMOVA). Results from these characterizations can help determine how well coriander accessions at the NCRPIS are representative of global diversity and provide various lines of evidence to validate existing classifications, or create a new one. In addition, this work can help reveal information about coriander's economically useful genetic variability for researchers and producers throughout the world (López et al. in press).

Materials and methods

Phenotypic diversity

Locations and experimental design

Data from a preliminary cycle of morphological and phenological characterization conducted in 2002 were used to eliminate redundant or contaminated accessions and to create a diverse, stratified sample of 60 accessions (Table 1) to serve as the basis for a second cycle of phenotypic characterization, which

Table 1 Coriander accessions tested in field trial during 2002 and 2003 in Ames, IA

Number	Accession	Country	Population code	LATDEC	LONDEC	ELEV (masl)
1	PI256061	Afghanistan	AF1_IIIb	34.517	69.183	1,808
2	Ames20047[a]	Armenia	AR1_IIb	40.290	44.942	NA
3	Ames24927[a]	Armenia	AR2_IIb	40.290	44.942	NA
4	Ames20046	Azerbaijan	AZ1_IIIb	40.371	49.893	−28
5	Ames24926[a]	Azerbaijan	AZ2_IIIb	40.290	47.536	NA
6	Ames24907	Bulgaria	BU1_IIIb	42.150	24.750	163
7	Ames18596	Canada	CA2_Ic	53.550	−113.500	643
8	PI483232	Chile	CHL1_IIIb	−33.450	−70.667	522
9	PI478378	China	CH2_IIIb	44.533	17.667	1,130
10	Ames18585	Czech Republic	CZ2_IIIb	49.200	16.633	212
11	PI193769	Ethiopia	ET1_Ic	9.100	37.250	2,143
12	PI193770	Ethiopia	ET2_Ic	9.100	37.250	2,143
13	Ames18563	France	FR1_IIIb	49.183	−0.350	5
14	Ames18564	France	FR2_IIIb	47.467	−0.550	40
15	Ames18565	Germany	GE2_IIIb	52.517	13.400	34
16	Ames18586	Germany	GE5_IIIb	52.517	13.400	34
17	Ames18591	Germany	GE6_IIIb	52.067	8.367	200
18	Ames24923[a]	Georgia	GEO1_IIIb	42.179	43.519	NA
19	Ames18507	India	IN7_Ib	28.667	77.217	215
20	Ames21101[a]	India	IN9_Ib	22.909	79.591	NA
21	Ames21104[a]	India	IN11_Ib	22.909	79.591	NA
22	Ames20048	Kazakhstan	KA3_IIIb	42.417	69.833	632
23	Ames26816	Mexico	ME1_IIIa	19.283	−98.438	2,200
24	Ames26817	Mexico	ME2_Ic	19.283	−98.438	2,200
25	Ames26819	Mexico	ME4_Ic	19.252	−98.482	2,200
26	Ames26820	Mexico	ME5_IIIa	19.258	−98.453	2,200

Table 1 continued

Number	Accession	Country	Population code	LATDEC	LONDEC	ELEV (masl)
27	Ames26822	Mexico	ME7_Ic	19.258	−98.453	2,200
28	Ames26829	Mexico	ME14_IIIa	19.042	−98.208	2,200
29	Ames18587	Netherlands	NT2_Ic	51.967	5.667	14
30	Ames23633	Oman	OM2_Ib	22.917	57.250	509
31	Ames23639	Oman	OM8_Ib	22.967	57.300	545
32	Ames23620	Pakistan	PA1_Ib	29.633	67.917	210
33	Ames18590	Poland	PO1_IIIb	52.250	21.000	94
34	Ames18595	Romania	RO1_IIIb	47.167	27.600	84
35	Ames18576	Russian Federation	RF2_IIIb	59.894	30.264	5
36	Ames18577	Russian Federation	RF3_IIIb	59.894	30.264	5
37	Ames18578	Russian Federation	RF4_IIIb	59.894	30.264	5
38	Ames18580	Russian Federation	RF5_IIIb	59.894	30.264	5
39	Ames21655[a]	Russian Federation	RF6_IIb	44.356	61.930	NA
40	Ames23626	Sudan	SU1_Ia	9.487	31.038	369
41	Ames23622	Syria	SY1_Ib	36.367	37.517	580
42	Ames23624	Syria	SY2_IIa	34.733	36.717	630
43	Ames25696	Syria	SY3_IIa	33.500	36.300	697
44	Ames13899	Tajikistan	TA1_IIIb	38.089	70.012	1,212
45	Ames13900	Tajikistan	TA2_IIIb	38.089	70.012	1,212
46	Ames4998[a]	Turkey	TU1_Ic	39.053	35.191	NA
47	PI171592	Turkey	TU2_Ic	39.983	41.683	1,774
48	PI172808	Turkey	TU3_IIIb	41.183	41.817	386
49	PI174129	Turkey	TU4_IIIb	39.017	43.350	1,669
50	PI174130	Turkey	TU5_IIIb	38.500	43.383	1,727
51	Ames18593	United Kingdom	UK2_IIIb	51.870	0.695	30
52	Ames18594	United Kingdom	UK3_IIIa	51.870	0.695	30
53	Ames24915	United States of America	US4_IIIa	34.283	−119.149	47
54	Ames24910	United States of America	US1_IIIb	34.283	−119.149	47
55	Ames24921	United States of America	US7_IIIa	34.283	−119.149	47
56	Ames25168	United States of America	US9_IIIb	42.035	−93.620	290
57	Ames25169	United States of America	US10_IIIb	42.035	−93.620	290
58	Ames25170	United States of America	US11_IIIa	42.035	−93.620	290
59	Ames24918	United States of America	US19_IIIb	34.283	−119.149	47
60	PI502320	Uzbekistan	UZ1_IIIb	40.433	67.967	273

LATDEC = Latitude in decimals; LONDEC = Longitude in decimals; ELEV = elevation in meters above sea level

[a] Geographical coordinates for these accessions were estimated based on the centroid for each country

NA = Data not available

was conducted during spring–summer 2003. In the 2003 trial, the experimental design was a complete randomized block design with two replications for each of two planting dates, April 23 and May 19. Both the 2002 and 2003 plots were located at the NCRPIS. The 2003 plot was located at 42°00′34.794″ North and 93°39′41.376″ West, at 243 meters above sea level (masl). According to the Soil Survey of Story County, Iowa (DeWitt 1984), the main soil types in these fields are Clarion and Nicollet loams, members of the Clarion–Webster–Nicollet association. For each field trial, the experimental units were four-row plots, 3.5 m long, spaced 0.5 m between rows; the seeding rate was 400 seeds per plot; and

plants were thinned to ~10 cm between plants within rows. Thinning was done when seedlings were ~10 cm tall (7–10 days after emergence).

Weather conditions during field evaluation

In 2002, conditions were relatively warm and dry during the evaluation period, and maximum temperatures reached at least 36°C with no temperatures below freezing. The 2003 growing season was much wetter and somewhat cooler on average, although this year registered higher maximum temperatures. The amount of precipitation for 2003 was 932 mm and 965 mm for first and second planting date, respectively; approximately twice that received in 2002. Differences in weather conditions for growing seasons for both years of evaluation may have contributed to higher overall yields for 2003. Photoperiod regimens were similar for both years and were computed with On-line Photoperiod Calculator software, version 1.95 EN (Lammi 2005).

Growing degree-days (GDD) were computed by the averaging method, with the formula:

$$\mathrm{GDD} = \left(\frac{T_{\min} + T_{\max}}{2}\right) - T_{\text{threshold}}$$

where GDD = Growing degree-days, $T_{\min}$ = Daily minimum temperature in °C, $T_{\max}$ = Daily maximum temperature in °C, $T_{\text{threshold}}$ = Threshold temperature = 10°C for coriander (Hayes 2006).

GDD showed a similar performance for both years; however, total accumulated growing-degree days were higher for 2002 (1,561) than for 2003 (1,146 and 1,357 for first and second planting date, respectively). Because of that, plants received sufficient growing-degree days for phenological changes more quickly in 2002 than in 2003.

Characteristics evaluated

We evaluated 19 phenological and morphological traits (Table 2), generally performed on the basis of protocols described by Diederichsen (1996) and Diederichsen and Hammer (2003). All these traits were measured on samples of ten individual plants randomly selected from the two central rows of each

Table 2 Phenological, morphological, and chemical traits evaluated in coriander populations in 2002 and 2003

Trait		Definition	Reference
Phenological			
1	Days to stem elongation (DSE)	Days from planting date to stem elongation for 50% of all plants in the two central rows of each plot	Diederichsen (1996)
2	Days to start of flowering (DSF)	Days from planting date to the first visible flower on each plant	Diederichsen (1996)
3	Range between days to stem elongation and days to start of flowering (RSDSF)	Computed with the formula: RSDSF = DSF − DSE	
4	Days to end of flowering (DEF)	Days from planting date to the last flower on each plant.	Diederichsen (1996)
5	Flowering range (FR)	Computed with the formula: DEF = DSF − DEF	
6	Days to harvest (DH)	Days from planting date to harvesting date.	Diederichsen (1996)
7	Days to maturity (DM)	Computed with the formula: DM = DH - DEF	
Morphological—vegetative			
8	Anthocyanins on the stem (ANST)	Before flowering, coloration along the stem, at the basal part and nodes.	Diederichsen (1996)
9	Number of basal leaves (NBL)	Total number of basal leaves, counted at the onset of flowering.	Diederichsen (1996)
10	Length of the longest basal leaf in cm (LLBL)	Measured at the onset of flowering.	Diederichsen (1996)
11	Blade shape of the longest basal leaf (BSBL)	At the onset of flowering, blade shape of the longest basal leaf was scored.	Diederichsen (1996)

Table 2 continued

Trait		Definition	Reference
12	Insertion angle of the longest basal leaf (HBL)	At the onset of flowering, arrangement of basal leaves in relation to the stem (prostrate versus erect) was scored.	Diederichsen (1996)
13	Plant height in cm (PH)	At the end of flowering, measured from the soil surface to the natural top of the plant	Diederichsen (1996)
Morphological—reproductive			
14	Anthocyanins in the petals (ANPE)	During flowering, petal coloration was scored.	Diederichsen (1996)
15	Number of umbels (NU)	Counted at harvest	
16	Plant seed yield (PY)	Computed with the formula: PY = TWF/10	
17	Percentage of split fruit (PSF)	Computed with the formula: PSF = (SFW/TFW) × 100	Diederichsen and Hammer (2003)
18	Weight of 1000 fruits in grams (W1000F)	A single sample of 500 whole fruits from a bulk from ten plants was weighed and multiplied by two	Diederichsen (1996)
19	Shape of the fruit (SF)	After fruit threshing and cleaning a bulk of fruits, fruit shape was scored.	Diederichsen (1996)
Chemical			
20	Essential oil content in percentage (EOCP)	Essential-oil content from 70 g of bulked and dried fruits was obtained by hydrodistillation	Diederichsen (1996)
21	Fatty acid content in percentage (FAC)	A 0.5 g sample of bulked, dried fruits was analyzed for fatty acids by using nuclear magnetic resonance spectroscopy.	Diederichsen (1996), Vlahov (1999)

plot. Two chemical traits related to seed composition, percentages of total essential oils and fatty acids, calculated on the basis of seed dry-weight, were also included in this study (Table 2), because of their importance in distinguishing among coriander populations in previous infraspecific classifications (Diederichsen and Hammer 2003).

Data analysis

Descriptive statistics were computed for the phenotypic data set, by combining data from both field evaluations, except for essential oil content (EOCP). Kurtosis and skewness measures were computed; all of those computations were carried out with SAS (SAS Institute 2002–2003). Variables with high values for kurtosis and skewness were considered to follow a non-normal distribution, and they were transformed by computing the square root (Cochran and Cox 1957).

All traits were also subjected to analysis of variance (ANOVA) by using SAS. Two ANOVAs were carried out. The first was done by combining data from 2002 and 2003, and sources of variation were years, planting dates within years (as replications), and accessions. The second ANOVA was solely done on the basis of 2003 data, with planting dates, blocks within planting dates, accessions, and the interaction of accessions × planting dates as sources of variation; finally, means for every accession were computed by using data from both experiments. Means for years and planting dates were compared by applying the *t*-test for paired comparisons (Cochran and Cox 1957).

Euclidean distances were computed with SAS, after standardizing means for each trait. Euclidean distances are used to estimate genetic distances between populations, based on quantitative morphological traits (Mohammadi and Prasanna 2003). Genetic Data Analysis Software (GDA) version 1.0 for Windows 95/NT (Lewis and Zaykin 2001) was used to construct a tree by applying the Unweighted Pair Method with Arithmetic Mean (UPGMA) algorithm to the matrix of Euclidean distances, and it was visualized by means of TreeView Software (Win 32) version 1.6.6 (Page 2001). Outcomes from the cluster analysis were then compared with Diederichsen and Hammer's (2003) infraspecific classification to identify relationships between both studies.

SAS was also used to construct a Pearson correlation matrix from the Euclidean distances, and a Principal Components Analysis was then conducted on the correlation matrix. The first three principal components were plotted on a three-dimensional graph.

Biochemical diversity

Seed essential-oil gas chromatography analysis

At seed maturity, 10 plants from every plot from the field evaluation were air-dried at 32°C, threshed, and cleaned; 70 g of dried, cleaned fruits were ground and used to extract the essential oil by hydrodistillation, following the protocol established by the Association of Official Agricultural Chemists (AOAC) (Woodbury 2000). Gas chromatography (GC) analysis was carried out on the extracted essential-oil samples as described in López (2006). Essential-oil extraction and GC analysis were done only once for each sample, but each accession was represented by four samples (two replications and two planting dates). The primary volatile compounds were identified by GC-Mass Spectrometry (MS) and confirmed by using authentic standards from Sigma-Aldrich (Milwaukee, WI). A Micromass GCT gas chromatograph-mass spectrometer (Waters Corp., Milford, MA) with a time-of-flight mass analyzer was used to confirm compound identities. Chemical characteristics for identified compounds were obtained from Flavor Works (1997–2000) Version 2.01 (Flavometrics, Inc., Anaheim Hills, CA), and retention indices were obtained from Flavornet (Acree and Arn 2004) to confirm compound identity.

Fatty-acid profile

Preparation of 2-ethyl-1-hexyl esters for GC analysis of extracted fatty acids was done as described by Isbell et al. (2006). GC was performed with a Hewlett-Packard 5890 Series II gas chromatograph equipped with a flame-ionization detector and an autosampler/injector, as described in López (2006).

Data analysis

Peak areas from essential-oil analyses were aligned based on retention times (Grob and Kaiser 2000). Peak areas from essential-oil analyses and fatty-acid percentages were weighted in relation to total essential-oil and fatty-acid production detected for the individual samples (López 2006). In this way, quantitative differences in overall production of the main volatile compounds and fatty acids present in each plot for every population (with one exception for seed essential oils) were analyzed.

The dataset from seed essential oils was subjected to ANOVA, with planting dates, replication within planting dates, population, and the interaction, population × planting date, as sources of variation. As fatty-acid analyses were carried out for samples from 2002 and 2003 field experiments, two ANOVAs were applied to analyze this data set; the first one was a combined analysis, with years, planting dates within years, and populations as sources of variation; the second ANOVA for fatty acids was similar to that employed for seed essential oils. ANOVAs were carried out with SAS. Population means were estimated from each of the two datasets; means for the main fatty acids were estimated with data from 2 years (2002 and 2003); and a third data matrix was constructed by joining the means from seed essential-oil analysis with those from fatty acids to carry out a combined seed-chemistry analysis for 60 accessions. Every population was assigned to a group based on the previous phenotypic classification. A Pearson correlation matrix was constructed for combined-mean data set and principal components analyses (PCA) were carried out by using SAS. The first three principal components were plotted on a three-dimensional graph. SAS was used to estimate Euclidean distance matrix from the combined dataset, and cluster analysis conducted for the three distance matrices by applying the UPGMA algorithm. Dendrograms for distances matrices were constructed by using Genetic Data Analysis Software (GDA) version 1.0 for Windows 95/NT (Lewis and Zaykin 2001) and visualized by means of TreeView Software (Win 32) version 1.6.6 (Page 2001).

Correlations among phenotypic, biochemical, and genetic distances

Mantel tests were carried out with GenAlEx Software for Excel (Peakall and Smouse 2006; Flanagan 2006) to compare previously calculated Euclidean distance matrices, based on phenotypic and biochemical data,

with the modified Rogers' distance matrix. The Mantel test is a sampled randomization technique that is used to determine associations between distance matrices (Sokal and Rohlf 1995).

Molecular analysis

DNA extraction

Extraction of genomic DNA was done individually for 12 randomly selected plants from each accession. Approximately 15–20 mg of leaf tissue powder from each plant were put into a 1.5 ml microfuge tube. A DNA-Easy™ Kit from Invitrogen™ (Carlsbad, CA, USA) was used for DNA extraction, following protocol 3 (Invitrogen™ Life Technologies 2003), with some modifications. After extraction, genomic DNA was resuspended in 100 µl of TE Buffer and stored at −80°C until DNA quantification. Extracted genomic DNA was standardized to 100 ng μl^{-1} and placed in 96-well plates stored at −80°C. Samples for amplified fragment length polymorphism (AFLP) reactions were prepared by bulking 20 µl of genomic DNA from three randomly selected plants from each accession. Four bulked samples of genomic DNA at 100 ng μl^{-1} were created for every accession.

AFLP reactions

AFLP procedures were conducted as described by Vos et al. (1995), with some modifications. We used two primer pairs for multiplexed selective amplifications, and an aliquot of 1.5 µl from each of the final selective amplification reactions, replicated twice, was submitted to the DNA Facility at Iowa State University for genotyping on an ABI Prism 3100 Genetic Analyzer, from Applied Biosystems (Foster City, CA, USA). A GeneScan 500 ROX Size Standard from Applied Biosystems was used to measure band size in base pairs.

Scoring

Files with genotyped data were imported to GenoGrapher software, version 1.6.0 (Benham 2001), for scoring as 1 or 0 for present or absent bands, respectively, as AFLPs are typically dominant molecular markers (Hartl 2000; Hartl and Jones 2001; Pompanon et al. 2005). A consensus binomial data matrix was then created with 240 columns representing 60 accessions and four sets, and a variable number of rows, specific to each primer pair, with rows representing the bins for bands of a specific size in base pairs on the gel.

Data analysis

A frequency matrix for AFLP markers, based on the presence or absence of reliable, polymorphic bands in each of the four bulks was constructed, with populations in rows and marker size in columns. On the basis of 80 informative markers greater than 70 base pairs in length with polymorphism information content (PIC) values greater than 0.15, determined by using Logiciel de Calcul de Distances Moléculaires entre Variétés (LCDMV) software (Lombard et al. 2001; Dubreuil et al. 2003), we computed Modified Rogers' distances for accessions under study, and a genetic distance matrix was then constructed by using Tools for Population Genetic Analyses Software (TFPGA) version 1.3 2000 (Miller 1997). Modified Rogers' distance is a Euclidean distance measure that considers each scored locus as an orthogonal dimension. It can be treated as a binomial variable, if random sampling is done among the genome (Mohammadi and Prasanna 2003). A dendrogram was constructed with modified Rogers' distances by applying the Neighbor-joining algorithm, which is widely used to infer both phylogenetic and phenetic relationships when working with sequence data or molecular markers (Hollingsworth and Ennos 2004).

In order to test the significance of the variation patterns obtained from phenotypic and biochemical dendrograms, an AMOVA was carried out, and statistics analogous to Wright's F_{ST} (Excoffier et al. 1992) were estimated by using Arlequin Software version 3.01 (Excoffier et al. 2006) on the 80 selected AFLP markers. Finally, four representative samples of plant material for each accession were taken from central rows of a 2004 garden established for this purpose; two samples were taken during the flowering period and the others taken after flowering finished. Those samples were prepared as vouchers and deposited at the Ada Hayden Herbarium in the Department of Ecology, Evolution and Organismal Biology at Iowa State University and at the United States National Arboretum Herbarium in Washington, DC.

Results and discussion

Phenotypic diversity

Variation

In the following discussion, each accession will be considered as a single population, defined as a group of intermating individuals from the same species that coincide together in time and place (Hartl 2000; Hedrick 2000; Hartl and Jones 2001; Bernardo 2002). In general, large variation in phenological, vegetative, reproductive, and chemical traits among populations was reflected by wide ranges for those characteristics (Table 3). Phenotypic variation for the NCRPIS coriander collection can be explained on the basis of the diverse ecogeographic origins of these accessions and local human selection focusing on the plant part of interest, leading to the possible formation of ecotypes and/or botanical varieties (Ivanova and Stoletova 1990; Diederichsen 1996; Diederichsen and Hammer 2003). It is important to highlight this finding because it is consistent with the goals of ex situ germplasm conservation (Spooner et al. 2005) for crop species and their wild relatives, preserving sufficient genetic and phenotypic diversity of important characteristics to support crop improvement and basic research.

Pearson correlations

Two morphological features, insertion angle of the longest basal leaf (HBL) and anthocyanins on the petals (ANPE), had the fewest significant correlations with other traits. Further, days to maturity (DM) also displayed few significant correlations with other characteristics and most of those were negative. Correlation between the two chemical traits was not significant, but those traits had interesting correlations with some of the phenological and morphological traits; for instance, EOCP was positively

Table 3 Basic statistics for phenotypic traits in coriander based on data from 2002 and 2003 field evaluations[a]

Variable	Maximum	Minimum	Range	Mean	Median	SD	SE	Skewness	Kurtosis
DSE	75.00	25.00	50.00	46.87	44.00	8.82	0.45	0.204	0.261
DSF	86.00	33.80	52.20	55.07	52.20	9.91	0.50	0.083	−0.051
RSEDSF	33.40	2.00	31.40	10.92	9.00	5.15	0.26	0.449	0.368
DEF	100.07	38.30	61.77	66.82	63.70	11.74	0.60	−0.178	−0.329
FR	26.50	3.30	23.20	11.75	10.10	4.04	0.20	0.346	−0.159
DH	115.00	59.00	56.00	91.37	87.00	10.94	0.61	−0.417	−0.361
DM[b]	60.70	9.0	51.70	23.28	23.90	6.10	0.34	0.423	1.495
ANST	9.00	1.00	8.00	3.14	2.10	1.96	0.10	1.081	0.480
NBL[b]	69.50	1.70	67.80	8.90	5.80	9.27	0.49	2.167	6.314
LLBL[b]	45.80	4.60	41.20	15.29	13.70	5.82	0.31	0.579	1.297
BSLBL	6.00	2.10	3.90	4.04	4.00	0.59	0.03	0.082	0.969
HBL	5.00	1.00	4.10	2.10	2.00	0.68	0.03	0.759	1.049
PH	91.30	20.10	71.20	51.36	46.85	15.15	0.81	0.034	−0.536
ANPE	5.40	1.00	4.40	1.94	1.70	0.98	0.05	1.237	1.000
NU	357.40	11.50	345.90	103.30	75.60	67.27	3.74	1.057	0.844
PSF	96.90	0.00	96.90	30.86	29.36	24.98	1.28	0.956	−0.050
PY	23.09	0.24	22.85	5.64	3.94	3.88	0.21	1.112	1.586
W1000F	13.34	2.22	11.12	5.52	5.84	2.07	0.10	1.083	1.120
SF	9.00	1.00	8.00	3.95	3.00	1.75	0.09	1.026	0.694
EOCP[a, b]	2.24	0.07	2.17	0.69	0.64	0.35	0.02	0.707	1.762
FAC	30.15	4.90	25.25	20.17	20.50	4.19	0.21	-0.611	0.558

[a] Statistics for EOCP were estimated based on data from 2003 field experiment, only

[b] Kurtosis and skewness presented in this table were estimated based on transformed data

correlated with plant yield (PY), but was negatively correlated with percentage of split fruit (PSF). This can be explained on the basis of volatilization of essential oils from the split fruits, where the oil ducts (Szujkó-Lacza 1994) are exposed. In addition, FAC had a positive correlation with shape of the fruit (SF) and with percentage of split fruit (PSF), but correlation with weight of 1,000 fruits (W1000F) was negative. Although high correlation between two variables cannot be necessarily considered as causal (Sokal and Rohlf 1995), it is interesting that in this study there were many strong correlations between phenological, morphological, and chemical traits. Special attention should be paid to the positive correlation between essential-oil content (EOCP) and plant yield (PY), because it means that it should be possible to increase the amount of seed produced and the concentration of essential oil simultaneously in a breeding program. Another interesting finding was the significant negative correlation between EOCP and SF, indicating a connection between high essential-oil content and rounded fruits, which are characteristic for subspecies *microcarpum*, as noted by Purseglove et al. (1981). However, we did not detect a significant correlation between EOCP and weight of 1,000 fruits, as has been described by Purseglove et al. (1981) for some coriander populations.

Analyses of variance

ANOVA detected significant differences between years for all phenotypic traits, except for number of basal leaves (NBL), and there were significant differences among the 60 selected populations for all these traits when combining data from both field evaluations (Table 4). From the analysis of data solely from the 2003 field evaluation, significant differences between planting dates were observed for 15 of the 21 phenological, morphological and chemical characteristics. Again, there were significant differences among populations for all of the traits, except for insertion angle of the HBL. Population × planting date interactions were significant for six of seven phenological traits, for five of six vegetative traits, but for only two of six reproductive characteristics (Table 4). In relation to chemical traits, there was a significant interaction only for FAC. It is important to mention the relevance of the interaction population × planting date for most of the phenotypic traits in this study; many authors have already pointed out the importance of the genotype-by-environment interaction for phenological and morphological traits in coriander (Jindla et al. 1985; Sastri et al. 1989; Bhandari and Gupta 1991; Angelini et al. 1997; Ali et al. 1999). However, many of the reproductive traits were relatively stable, and the lack of a significant interaction for essential-oil content highlights its importance in the classification and characterization of coriander populations (Diederichsen 1996; Diederichsen and Hammer 2003).

Comparison of means between years and between planting dates

The 2003 growing season resulted in better vegetative development and higher plant yield. Values measured for the chemical traits were also higher in 2003 (Table 5). Means for the first planting date were higher than for the second planting date for four of seven phenological traits (Table 5); only the value for range between stem elongation and days to start of flowering (RSEDSF) was higher for the second planting date. The second planting date generally resulted in a more compressed developmental cycle and reduced values for some reproductive characteristics, including plant yield (PY) and weight of 1,000 fruits (W1000F). Essential-oil content (EOCP) was lower in seeds harvested from the second planting date, whereas FAC was higher. Differences between phenological, morphological, and chemical traits due to years and planting dates in this study demonstrate the effect of the environment on the expression of many of these phenotypic traits (Lombard et al. 2001; Nuel et al. 2001). It confirms the capacity of the plants to modify trait expression, as a consequence of environmental changes; this phenomenon is called phenotypic plasticity (DeWitt and Scheiner 2004). Although plasticity is a desirable attribute for plant adaptation, it confounds population characterization in genebanks (Lombard et al. 2001; Nuel et al. 2001; Bhat et al. 2004). Thus, it is important to identify the most stable traits for morphological characterization.

Certain characters, such as days to maturity (DM), blade shape of the longest basal leaf (BSLBL), insertion angle of the longest basal leaf (HBL), anthocyanins on the petals (ANPE), number of umbels (NU), and shape of the fruits (SF), showed more stability between planting dates; furthermore, percentage of split fruit

Table 4 Mean squares for phenotypic traits in coriander. ANOVA for combined data for years 2002 and 2003 and for two planting dates in 2003

Variable	Combined analysis			2003 Analysis	
	Years	Populations	Planting Dates	Populations	Interaction Population × Planting Date
DSE	3718.37***	320.45***	1770.96***	231.26***	17.01***
DSF	4313.43***	461.64***	1150.92***	327.56***	24.52***
RSEDSF	1920.57***	35.66***	66.55**	24.56**	13.11*
DEF	8041.34***	627.02***	2238.90***	424.24***	21.60**
FR	575.86***	38.12***	179.40***	34.19**	16.84***
DH	343.25***	501.60***	2541.41***	403.18***	32.70***
DM[a]	3.79***	0.43**	0.19 ns	0.32*	0.20 ns
ANST	136.21***	14.67***	15.46***	14.02***	1.52*
NBL[a]	1.49 ns	4.95***	20.45***	4.02***	0.73***
LLBL[a]	0.82*	1.78***	12.84***	1.14***	0.11***
BSLBL	5.14***	1.34***	0.01 ns	1.32***	0.19 ns
HBL	1.61*	0.78***	0.19 ns	0.87 ns	0.59**
PH	12169.09***	590.85***	11156.65***	511.23***	79.85***
ANPE	5.19*	1.69***	1.94 ns	1.45*	0.89*
NU	33702.16***	15598.93***	1.72 ns	14166.94***	2416.28*
PSF	48399.56***	2097.39***	841.74**	1053.35***	89.25 ns
PY	195.87***	31.47***	388.11***	32.86***	9.22 ns
W1000F	486.97***	15.33***	18.53***	7.38***	0.32 ns
SF	8.56**	13.88***	2.08 ns	9.79***	0.98 ns
EOCP[a]	NE	NE	0.03*	0.14***	0.01 ns
FAC	606.37***	45.44***	209.77***	20.58***	7.87*

ns = Non-significant

* Significant ($\alpha = 0.05$)

** Highly significant ($\alpha = 0.01$)

*** Very highly significant ($\alpha = 0.001$)

NE = not evaluated

[a] Mean squares based on transformed data

(PSF), plant yield (PY), weight of 1000 fruits (W1000F), shape of the fruit (SF), and essential-oil content (EOCP) showed a lack of interaction with planting dates, and thus, those traits can be considered as stable and most appropriate to score for future characterization of coriander. In addition, soil characteristics, maximum and minimum temperatures, growing-degree days, precipitation, and photoperiod for the growing season, are important environmental characteristics to record as part of the characterization process.

Cluster analysis and phenetic relationships

A dendrogram showing phenetic relationships among studied populations as determined from 21 phenotypic traits (Table 2) is presented in Fig. 1. At least three main groups can be distinguished in this tree. Group I includes 17 populations, which can be subdivided into three subgroups: subgroup Ia is formed only by one population from Sudan; subgroup Ib includes populations from India, Oman, Pakistan, and Syria; subgroup Ic contains populations from Canada, Ethiopia, Mexico, the Netherlands, and Turkey (Fig. 1). Group II, located between Groups I and III, is the smallest, with only five populations; it can be divided into subgroups IIa and IIb. Subgroup IIa contains only populations from Syria, and subgroup IIb includes populations from Armenia and the Russian Federation (Fig. 1). Group III is the largest group encompassing 28 populations; this group is divided into subgroups IIIa

Table 5 Year and planting date means comparisons for phenotypic traits in coriander by using the *t*-test

Variable	Year means		Planting date means	
	2002	2003	23 April 2003	19 May 2003
DSE	42.73 b	49.30 a	52.02 a	46.59 b
DSF	50.58 b	57.71 a	59.90 a	55.52 b
RSEDSF	7.84 b	12.73 a	7.88 b	8.93 a
DEF	60.67 b	70.44 a	73.50 a	67.39 b
FR	10.10 b	12.73 a	13.60 a	11.87 b
DH	89.01 b	92.18 a	95.43 a	88.92 b
DM	27.74 a	21.74 b	21.93 a	21.54 a
ANST	2.33 b	3.62 a	3.36 b	3.87 a
NBL	7.18 b	9.68 a	7.22 b	12.15 a
LLBL	15.56 a	15.17 a	16.95 a	13.40 b
BSLBL	3.89 b	4.13 a	4.12 a	4.14 a
HBL	2.00 b	2.16 a	2.13 a	2.19 a
PH	41.02 b	55.97 a	62.79 a	49.16 b
ANPE	2.06 a	1.88 b	1.97 a	1.79 a
NU	79.70 b	111.38 a	111.29 a	111.46 a
PSF	46.17 a	21.88 b	20.00 b	23.75 a
PY	3.22 b	6.47 a	7.74 a	5.20 b
W1000F	6.98 a	4.67 b	4.95 a	4.39 b
SF	3.74 b	4.08 a	3.98 a	4.17 a
EOCP	NE	0.69	0.71 a	0.68 b
FAC	18.54 b	21.13 a	20.19 b	22.06 a

Means with same letters in rows are not statistically different, with $\alpha = 0.05$

NE = not evaluated

and IIIb. Subgroup IIIa is formed of populations from Mexico, United Kingdom, and the United States of America. Subgroup IIIb is the largest and most diverse subgroup, based on the geographical origins of the populations that it includes; those populations are from Afghanistan, Azerbaijan, Bulgaria, Chile, China, Czech Republic, France, Georgia, Germany, Kazakhstan, Poland, Romania, Russian Federation, Tajikistan, Turkey, United Kingdom, the United States of America, and Uzbekistan (Fig. 1).

As both subgroups Ic and IIIb are formed of geographically diverse arrays of populations, they raise interesting questions that lead us to pose two hypotheses that may underly these groups. First, geographic diversity within the subgroups could reflect the widespread sharing of populations through international trade. Second, this diversity could result from multiple evolutionary origins for these groups, related to human selection for similar traits in different regions. We discuss these hypotheses as part of our molecular analysis below.

Principal components analysis

The four first principal components had eigenvalues greater than 1.0, and, together, they explained 73.5% of the total variation for this assemblage of phenotypic traits. The first principal component had an eigenvalue of 9.4, explaining 45.0% of the whole variation. Phenological traits, such as days to stem elongation (DSE), days to start of flowering (DSF), days to end of flowering (DEF), and days to harvest (DH), contributed strongly to variation for this principal component, with eigenvectors above 0.30. The second principal component's eigenvalue was 2.7; it explained 13.0% of the total variation. Reproductive traits, such as shape of the fruit (SF), percentage of split fruit (PSF), and plant yield (PY), and the chemical trait, percentage of essential oil (EOCP), contributed strongly to the variation of this principal component, with eigenvectors above 0.30. The third principal component's value was 2.0; it explained 9.6% of the total variation. Traits with eigenvectors above 0.30 that contributed strongly to its variation included all four classes of traits, phenological, vegetative, reproductive, and chemical, with flowering range (FR), number of basal leaves (NBL), anthocyanins on the petals (ANPE), and FAC, respectively. The fourth principal component had 1.2 as its eigenvalue, it explained 5.9% of the total variation, and the phenological trait, days to maturity (DM), was significantly related to this principal component, explaining most of its variation, with an eigenvector value of 0.75. Figure 2 shows a three-dimensional arrangement of phenotypic data for selected coriander populations based on the first three principal components, which collectively explained 67.6% of the total variation. Patterns revealed by cluster analysis were confirmed by principal components analysis; populations from Group I form well-defined subgroups Ib (star) and Ic (pyramid), and a single, distinct population, coded as Ia (cube). Populations from Group II include two well-defined subgroups, IIa (square) and IIb (club). Populations from Group III are divided into two subgroups, a well-defined IIIa subgroup (flag) and a large, dispersed IIIb subgroup (balloon).

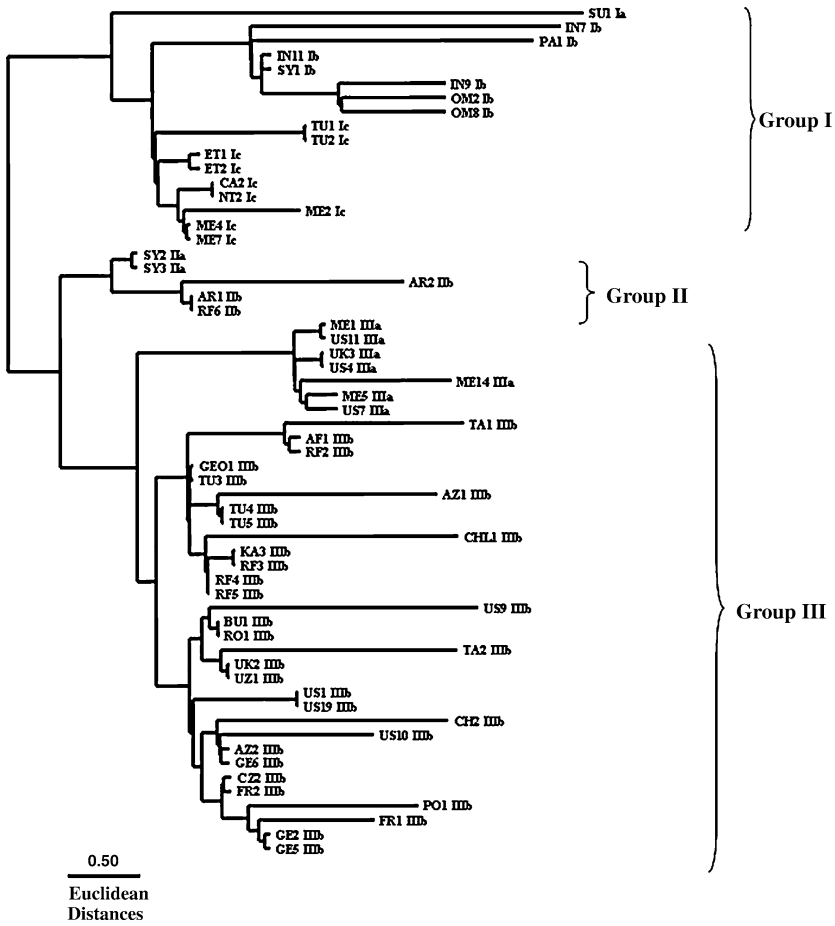

Fig. 1 Phenetic relationships among 60 coriander populations based on Euclidean distances from phenotypic data, presented as a dendrogram

Descriptions of the defined groups

Plants belonging to Group I have the shortest juvenile period and three or fewer basal leaves; their basal leaves are short, as are the plants. These plants have few umbels and low seed yield, but with large, heavy ovate fruits. The seeds are intermediate in essential-oil content but with the highest fatty-acid content. Populations from Group I conform to descriptions of two subspecies, *sativum* and *indicum*, as described by Diederichsen and Hammer (2003). Subgroup Ib populations fit with subsp. *indicum*, and populations from subgroup Ic are representative of subsp. *sativum*, with their distinctive, large fruits; this subspecies is found in the Mediterranean, western Europe and in America. A discrepancy with the classification of Diederichsen and Hammer (2003) relates to their proposed botanical variety *pygmaeum* Stolet. ex Diederichsen, which was included as part of subsp. *indicum*. However, in the present study, population SU1_Ia, which fits all the characteristics for var. *pygmaeum*, appears as unique sample not nested within subgroup Ib, the subgroup which conforms best to the description of subsp. *indicum*.

Group II is formed by populations of plants with very long juvenile periods and many, large basal leaves; the plants are among the tallest with many umbels and low to intermediate seed yields. Their fruits are very small, low in weight, with a rounded shape and very low essential-oil and low fatty-acid content. Subgroup IIa is composed by populations from Syria, and they may belong to subspecies *microcarpum* var. *syriacum*, although in our study they are not included in group III, which corresponds to this subspecies (Fig. 1). Regarding subgroup IIb, there is no clear correspondence between it and any of the subspecies previously described by Diederichsen and Hammer (2003); Group IIb populations have

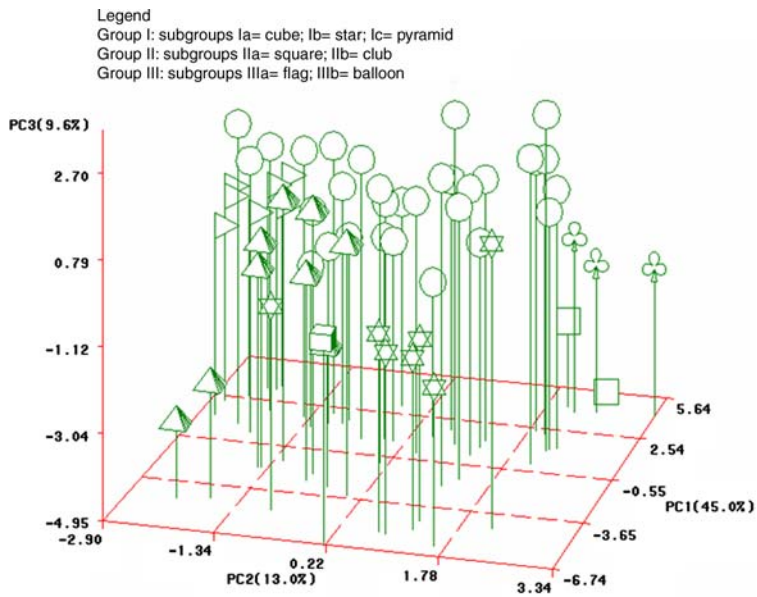

Fig. 2 Phenetic relationships among 60 coriander populations based on the first three principal components from phenotypic data, presented as a three-dimensional scatter plot

traits intermediate between subspecies *indicum* and subspecies *microcarpum* DC., and they might even represent hybrids between those subspecies.

Plants from Group III are intermediate in juvenility, with more than four basal leaves; their basal leaves are intermediate in length, and the plants are taller than are populations from Group I. These plants have an intermediate to high number of umbels, with the highest seed yields and rounded, small fruits, intermediate in weight. This group has the highest essential-oil content and intermediate fatty-acid content. Populations from Group III correspond closely to subsp. *microcarpum*, well described by Diederichsen and Hammer (2003), with subgroup IIIa all conforming to var. *microcarpum*, and subgroup IIIb encompassing a poorly differentiated assemblage of three of Diederichsen and Hammer's (2003) varieties.

All three subspecies proposed by Diederichsen and Hammer (2003) are represented among the accessions evaluated in this study (Table 6). Below the subspecific level, our accessions match the descriptions of at least nine of the ten botanical varieties previously described by the abovementioned authors. Of the four botanical varieties that Diederichsen and Hammer (2003) included under subspecies *indicum*, only var. *bhutanense* Diederichsen seems to be missing from our subset of 60 accessions; however, from our preliminary set of 139 accessions, accession Ames 23619 from Bhutan may be a representative of this botanical variety. Subgroup IIb does not fit descriptions for any previously proposed subspecies. The four varieties of subspecies *microcarpum* are problematic; one of them clusters in subgroup IIa and the other three cluster in Group III. Images of vouchers from accessions representative of the previously described subspecies and botanical varieties are shown in López (2006).

Biochemical diversity

Identification of seed essential-oil components

From 104 scored retention times, 35 primary volatile compounds were identified in seed essential oil samples. Identified volatile compounds are listed in Table 7, with data on percentage of fruit weight and relative abundance. Linalool was the most abundant compound, on average accounting for more than 70% of the total essential oil. In decreasing order of abundance, other important compounds were α-pinene, geranyl acetate, camphor, γ-terpinene, terpinolene, citronellol, geraniol, and limonene. The predominant compounds detected in this study agree with those reported by other researchers for coriander essential oil (Purseglove et al. 1981; Formáček and Kubeczka 1982; Kerrola and Kallio 1993; Frank et al. 1995; Diederichsen 1996; Jeliazkova et al. 1997; Bandoni et al. 1998; Baratta et al. 1998; Misharina 2001;

Table 6 Comparison of 60 coriander accessions from NCRPIS, with infraspecific taxa, as proposed by Diederichsen and Hammer (2003)

Number	Accession	Code for population	Subspecies	Variety
40	Ames 23626	SU1_Ia	*indicum*	*pygmaeum*
19	Ames 18507	IN7_Ib	*indicum*	*indicum*
20	Ames 21101	IN9_Ib	*indicum*	*indicum*
21	Ames 21104	IN11_Ib	*indicum*	*indicum*
30	Ames 23633	OM2_Ib	*indicum*	*omanense* Diederichsen
31	Ames 23639	OM8_Ib	*indicum*	*omanense*
32	Ames 23620	PA1_Ib	*indicum*	*indicum*
41	Ames 23622	SY1_Ib	*indicum*	*indicum*
7	Ames 18596	CA2_Ic	*sativum*	*africanum* Stolet.
11	PI 193769	ET1_Ic	*sativum*	*africanum*
12	PI 193770	ET2_Ic	*sativum*	*africanum*
24	Ames 26817	ME2_Ic	*sativum*	*sativum*
25	Ames 26819	ME4_Ic	*sativum*	*sativum*
27	Ames 26822	ME7_Ic	*sativum*	*sativum*
29	Ames 18587	NT2_Ic	*sativum*	*africanum*
46	Ames 4998	TU1_Ic	*sativum*	*sativum*
47	PI 171592	TU2_Ic	*sativum*	*sativum*
42	Ames 23624	SY2_IIa	*microcarpum*	*syriacum* Diederichsen
43	Ames 25696	SY3_IIa	*microcarpum*	*syriacum*
2	Ames 20047	AR1_IIb	intermediate	undefined
3	Ames 24927	AR2_IIb	intermediate	undefined
39	Ames 21655	RF6_IIb	intermediate	undefined
23	Ames 26816	ME1_IIIa	*microcarpum*	*microcarpum*
26	Ames 26820	ME5_IIIa	*microcarpum*	*microcarpum*
28	Ames 26829	ME14_IIIa	*microcarpum*	*microcarpum*
52	Ames 18594	UK3_IIIa	*microcarpum*	*microcarpum*
53	Ames 24915	US4_IIIa	*microcarpum*	*microcarpum*
55	Ames 24921	US7_IIIa	*microcarpum*	*microcarpum*
58	Ames 25170	US11_IIIa	*microcarpum*	*microcarpum*
1	PI 256061	AF1_IIIb	*microcarpum*	*microcarpum*
4	Ames 20046	AZ1_IIIb	*microcarpum*	*asiaticum* Stolet.
5	Ames 24926	AZ2_IIIb	*microcarpum*	*microcarpum*
6	Ames 24907	BU1_IIIb	*microcarpum*	*microcarpum*
9	PI 478378	CH2_IIIb	*microcarpum*	*asiaticum*
8	PI 483232	CHL1_IIIb	*microcarpum*	*vavilovii* (Stolet.) Diederichsen
10	Ames 18585	CZ2_IIIb	*microcarpum*	*asiaticum*
13	Ames 18563	FR1_IIIb	*microcarpum*	*asiaticum*
14	Ames 18564	FR2_IIIb	*microcarpum*	*microcarpum*
15	Ames 18565	GE2_IIIb	*microcarpum*	*microcarpum*
16	Ames 18586	GE5_IIIb	*microcarpum*	*microcarpum*
17	Ames 18591	GE6_IIIb	*microcarpum*	*microcarpum*
18	Ames 24923	GEO1_IIIb	*microcarpum*	*asiaticum*
22	Ames 20048	KA3_IIIb	*microcarpum*	*microcarpum*

Table 6 continued

Number	Accession	Code for population	Subspecies	Variety
33	Ames 18590	PO1_IIIb	*microcarpum*	*microcarpum*
34	Ames 18595	RO1_IIIb	*microcarpum*	*microcarpum*
35	Ames 18576	RF2_IIIb	*microcarpum*	*vavilovii*
36	Ames 18577	RF3_IIIb	*microcarpum*	*vavilovii*
37	Ames 18578	RF4_IIIb	*microcarpum*	*asiaticum*
38	Ames 18580	RF5_IIIb	*microcarpum*	*vavilovii*
44	Ames 13899	TA1_IIIb	*microcarpum*	*vavilovii*
45	Ames 13900	TA2_IIIb	*microcarpum*	*microcarpum*
48	PI 172808	TU3_IIIb	*microcarpum*	*asiaticum*
49	PI 174129	TU4_IIIb	*microcarpum*	*vavilovii*
50	PI 174130	TU5_IIIb	*microcarpum*	*vavilovii*
51	Ames 18593	UK2_IIIb	*microcarpum*	*microcarpum*
54	Ames 24910	US1_IIIb	*microcarpum*	*asiaticum*
56	Ames 25168	US9_IIIb	*microcarpum*	*microcarpum*
57	Ames 25169	US10_IIIb	*microcarpum*	*microcarpum*
59	Ames 24918	US19_IIIb	*microcarpum*	*asiaticum*
60	PI 502320	UZ1_IIIb	*microcarpum*	*asiaticum*

Smallfield et al. 2001; Gil et al. 2002). In addition, mean percentages for compounds, based on their presence in essential oil samples (Table 7), generally agree with earlier reports; Diederichsen and Hammer (2003) highlighted linalool, camphor, limonene, and myrcene as very useful compounds for infraspecific classification in coriander. Moreover, Table 7 displays the wide variation among studied populations in the relative amounts of the main compounds, which suggests that many different aroma types could be selected or bred from these populations. For instance, we have identified populations with relatively low linalool content (~49% in SY2_IIa) and those with elevated levels of α-pinene (~9% in IN9_Ib), geranyl acetate (~8.5% in IN11_Ib), geraniol (~7% in SY2_IIa), γ-terpinene (~4% in US7_IIIa), terpinolene (~6% in SY2_IIa), ß-terpineol (~6% in SY2_IIa), and camphor (~6% in ET2_Ic); concentrations many times higher than threshold levels for human detection (Leffingwell and Associates 1999) (Table 7).

Identification of the main fatty acids

Results of our seed fatty-acid analysis revealed that, in decreasing order of abundance, petroselenic, linoleic, oleic, palmitic, stearic, vaccenic, and octadecenoic acids were the most common fatty acids, together accounting for 99% of the total (Table 8). For purposes of analysis, all other minor, unidentified components were grouped together in an eighth category called ''others.'' Other authors have reported similar fatty-acid profiles in coriander, with petroselenic acid as the major constituent and linoleic acid as the second most abundant constituent (Kleiman and Spencer 1982; Ross and Murphy 1992; Angelini et al. 1997; Ramadan and Mörsel 2003). Significant variation in content of all the identified fatty acids was observed, highlighting the chemical diversity in fatty-acid composition among these populations; similar results for infraspecific variation in fatty-acid profiles for other species have been reported (Miller et al. 1965; Graham 1989; Davik and Heneen 1993; Alemayehu and Becker 2001; Salywon et al. 2005). In contrast, Knapp et al. (1991) reported only limited diversity in fatty acids at the infraspecific level in *Cuphea viscosissima* Jacq.

Analyses of variance

Table 9 summarizes an ANOVA for essential-oil compounds (as reflected in distinct retention times), displaying the proportions of the volatile compounds that had significance for every source of variation:

Table 7 Mean percentages of the main volatile compounds identified in coriander seed essential oil from 60 populations

Retention time (SPB-1000)[a]	Compound	Percentage (in fruits)[b]	Percentage (in oil)[c]			Odor threshold as percentage[d] NA
			Mean	Max	Min	
6.169	α-thujene	0.0003	0.06	0.17	0.00	NA
6.627	α-pinene	0.0250	3.71	9.14	1.97	0.0000006
7.518	camphene	0.0030	0.34	0.75	0.02	NA
7.954	hexanal	0.0000	0.01	0.02	0.00	0.00000045
8.362	β-pinene	0.0030	0.41	1.07	0.25	0.000014
8.628	sabinene	0.0010	0.18	0.32	0.04	NA
9.079	myrcene	0.0003	0.05	0.15	0.00	0.0000013
9.416	phellandrene	0.0030	0.38	0.71	0.09	NA
9.918	heptanal	0.0004	0.06	0.20	0.01	0.0000003
10.145	limonene	0.0060	0.79	1.60	0.04	0.000001
10.328	eucalyptol	0.0003	0.06	1.04	0.00	0.0000012
11.062	γ-terpinene	0.0150	2.01	3.84	0.20	NA
11.590	terpinolene	0.0140	2.22	5.85	0.33	0.00002
11.834	octanal	0.0010	0.12	0.28	0.00	0.00000007
15.570	nonanal	0.0010	0.16	0.35	0.03	0.0000001
16.304	1-octen-3-ol	0.0009	0.14	0.49	0.03	0.0000001
16.915	linalool oxide	0.0050	0.88	2.79	0.15	NA
17.497	decanal	0.0001	0.03	0.10	0.00	0.00000001
17.786	camphor	0.0210	2.50	5.67	0.07	0.0001
18.347	linalool	0.4950	71.21	83.15	49.29	0.0000006
18.536	octanol	0.0010	0.16	0.36	0.08	0.0000011
19.641	myrtenal (t)[e]	0.0010	0.23	0.74	0.06	NA
19.847	2-decenal	0.0010	0.20	0.36	0.06	0.00000003
20.867	β-terpineol (t)[e]	0.0060	1.04	5.90	0.18	0.000033
21.070	α-humulene	0.0001	0.03	0.23	0.00	NA
21.190	neral (t)[e]	0.0001	0.03	0.15	0.00	NA
21.968	borneol (t)[e]	0.0005	0.09	0.20	0.04	NA
22.045	α-terpinyl acetate	0.0005	0.07	0.16	0.00	NA
22.868	neryl acetate	0.0003	0.05	0.09	0.01	NA
23.329	citral	0.0010	0.12	0.23	0.05	0.000003
23.769	geranyl acetate (t)[e]	0.0240	3.52	8.59	1.03	0.0000009
25.116	myrtenol	0.0006	0.08	0.19	0.03	NA
25.486	nerol	0.0020	0.29	1.45	0.00	0.00003
26.408	citronellol	0.0140	1.83	3.57	0.60	0.000004
26.675	geraniol (t)[e]	0.0070	1.37	7.37	0.14	0.000004

[a] Column used for GC analysis

[b] Weighted by the essential oil content (%) in fruits

[c] Estimated as percentage of the volatile compound in the essential oil sample

[d] Minimum odor threshold in water (Leffingwell and Associates 1999), accessed on line at http://www.leffingwell.com/odorthre.htm

[e] Tentatively identified

Max = maximum; Min = minimum; NA = not available

Table 8 Amount and percentage of main fatty acids identified in 60 populations of coriander

Fatty acid	Percentage (in fruits)[a]	Percentage[b] (in oil)		
		Mean	Maximum	Minimum
Palmitic	0.936	4.59	5.61	3.05
Stearic	0.432	2.60	5.45	1.29
Octadecenoic	0.889	0.39	2.13	0.00
Petroselenic	13.881	66.75	73.14	61.72
Oleic	1.807	8.87	10.57	7.16
Vaccenic	0.238	1.10	1.55	0.34
Linoleic	3.044	14.61	16.51	12.37
Others	0.200	0.89	2.01	0.00

[a] Weighted by the fatty-acid content (%) in fruits

[b] Estimated as percentage of the fatty acid in the oil sample

Table 9 Statistically significant and non-significant (at the $P = 0.05$ level) mean squares for retention times from seed essential-oil GC analyses in coriander; samples obtained in 2003

Source of variation	Essential oil		
	Significant	Non-significant	Total
Planting Date (PD)	97 (93.27%)	7 (6.73%)	104
Population (PO)	52 (50.00%)	52 (50.00%)	104
Interaction PD × PO	10 (9.62%)	94 (90.38%)	104

planting date, population, and the interaction of population × planting date. Planting-date differences were significant for a high proportion of the seed essential-oil compounds, and significant differences among populations were detected for half of the scored compounds. In contrast, significant interactions were detected in less than 10% of the seed essential oils. Thus, these ANOVA results support past research (Purseglove et al. 1981; Angelini et al. 1997; Argañosa et al. 1998) that documented significant environmental effects on the constituents of the seed essential oils in coriander. However, for diversity studies designed to produce stable systematic classifications, it is desirable that traits be relatively unaffected by the environment (Lombard et al. 2001; Nuel et al. 2001).

Differences between years were detected for all the fatty acids in the profile; among populations, all components except octadecenoic acid and the ''others'' class showed significant differences (Table 10). From the ANOVA for 2003 data, five of eight fatty acids showed differences for planting date and for populations. Only stearic acid showed a significant interaction between populations and planting date. Most of the fatty acids were affected by the environment (i.e., the planting dates), although the relative unimportance of the population × planting date interaction indicates that these compounds (and more than 90% of the volatile oil components) should be reliable traits for classification. These phenomena are consistent with other reports describing the advantages of using fatty acids for taxonomic classification in plant species (Miller et al. 1965; Davik and Heneen 1993; Granot et al. 1996; Mayworm and Salatino 2002).

Table 10 Mean squares from ANOVA for the main fatty acids in coriander seeds; samples produced in 2002 and 2003

Fatty acid	Combined			2003	
	Years	Population (PO)	Planting Date (PD)	Population (PO)	Interaction PD × PO
Palmitic	2.73***	0.07**	0.298***	0.035***	0.009 ns
Stearic[a]	10.61***	0.02***	0.002 ns	0.005***	0.001*
Octadecenoic[a]	2.40***	0.04 ns	0.064 ns	0.058 ns	0.063 ns
Petroselenic	403.66***	28.07***	92.779***	14.297***	4.794 ns
Oleic	0.61***	0.45***	0.320*	0.231***	0.092 ns
Vaccenic[a]	1.32***	0.04***	0.077***	0.007 ns	0.006 ns
Linoleic	48.04***	0.86***	4.804***	0.507***	0.137 ns
Others[a]	4.89***	0.04 ns	0.021 ns	0.046 ns	0.036 ns

[a] Mean squares from transformed data (square root)

ns = non-significant

* Significant ($\alpha = 0.05$)

*** Very highly significant ($\alpha = 0.001$)

Cluster analysis

Cluster analyses based on the combined essential-oil and fatty-acid data (Fig. 3) defined five clusters and two single populations. But this dendrogram shows only weak relationships with clustering patterns obtained from phenotypic traits. To its credit, the dendrogram in Fig. 3 does bring together some groups of morphologically similar populations and others formed of populations with proximal geographical origins. For example, Group 1 in Fig. 3 is made up only of populations from the same morphological subgroup (IIIb), and Group 3 includes consistent clusters of seven populations from morphological subgroup IIIb and five from subgroup IIIa. There were also four very tight pairs from the same country of origin, with one of those pairs including members of two different morphological groupings from Turkey: TU2_Ic and TU5_IIIb.

Descriptions of biochemical groups

Summaries of the chemical profiles for five groups defined on the basis of the combined analysis of essential-oil and fatty-acid data are presented in Table 11, together with two single populations that did not fit with any group. Group 1 is characterized by the highest percentages of linalool, camphene, and camphor, and the second highest percentage for limonene. In addition, this group had the highest percentages for oleic and linoleic acids. Group 2 had the highest percentages for nonanal and geranyl acetate, and the second highest percentages for linalool, α-humulene, and geraniol. This group had the highest content for palmitic and stearic acids. Group 3 presented the highest proportions for sabinene, phellandrene, limonene, γ-terpinene, terpinolene, and citronellol, along with the second highest percentages for camphor and for stearic and linoleic

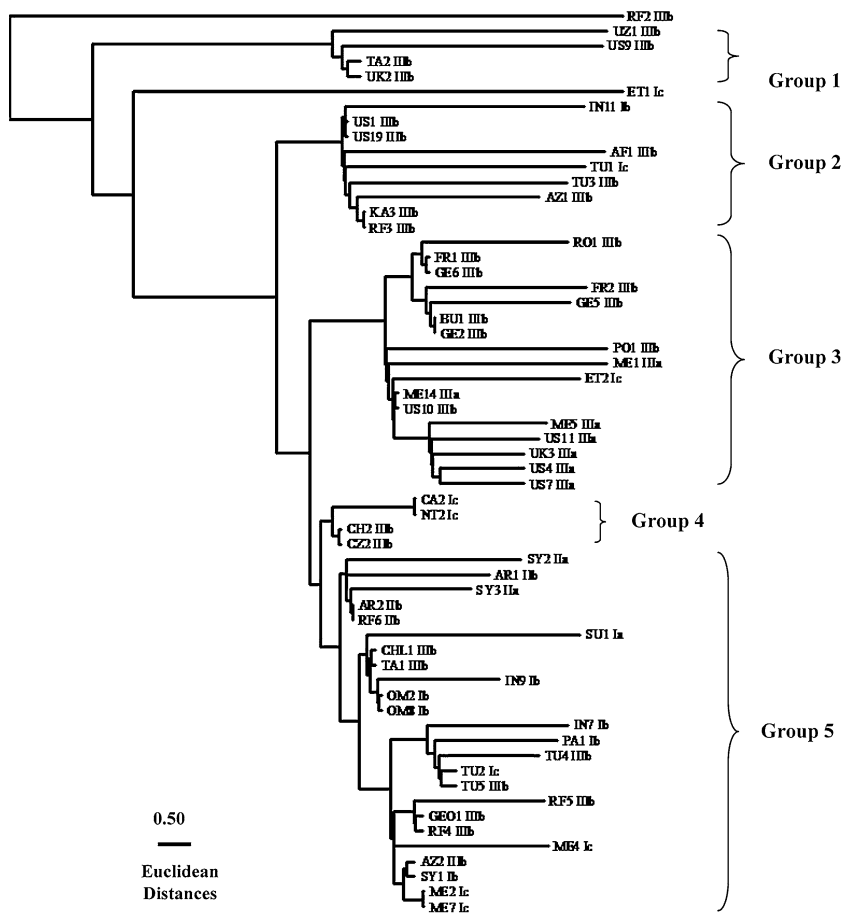

Fig. 3 Phenetic relationships among 60 coriander populations based on Euclidean distances from biochemical data, presented as a dendrogram

Table 11 Means in percentages for main volatile compounds and fatty acids for groups formed on the basis of combined essential-oil and fatty-acid profiles in coriander

Component	Group 1	Group 2	Group 3	Group 4	Group 5	RF2_IIIb	ET1_Ic
Volatile compounds							
α-thujene	0.01	0.06	0.04	0.06	0.07	0.05	0.05
α-pinene	3.49	3.41	3.60	3.95	3.92	4.05	3.33
camphene	0.55	0.19	0.51	0.43	0.22	0.04	0.45
hexanal	0.00	0.01	0.00	0.01	0.01	0.01	0.01
β-pinene	0.33	0.38	0.38	0.44	0.46	0.46	0.38
sabinene	0.21	0.12	0.24	0.22	0.16	0.06	0.12
myrcene	0.01	0.05	0.03	0.04	0.06	0.05	0.04
phellandrene	0.51	0.24	0.52	0.41	0.32	0.16	0.33
heptanal	0.02	0.07	0.04	0.13	0.06	0.10	0.20
limonene	1.16	0.46	1.19	0.97	0.55	0.13	1.04
eucalyptol (1,8-cineole)	0.06	0.02	0.06	0.05	0.07	0.01	0.03
γ-terpinene	2.57	1.06	2.79	2.43	1.76	0.38	0.66
terpinolene	1.57	1.37	2.72	2.66	2.35	0.33	1.46
octanal	0.22	0.04	0.19	0.15	0.07	0.01	0.11
nonanal	0.10	0.21	0.14	0.16	0.16	0.14	0.20
1-octen-3-ol	0.08	0.22	0.10	0.12	0.16	0.13	0.27
linalool oxide	0.28	1.03	0.46	1.90	0.97	1.49	2.79
decanal	0.00	0.03	0.01	0.07	0.03	0.05	0.10
camphor	4.56	1.29	4.34	2.15	1.35	0.28	3.72
linalool	73.98	72.73	71.77	63.33	71.54	69.67	62.59
octanol	0.12	0.15	0.15	0.15	0.18	0.23	0.11
myrtenal	0.12	0.30	0.13	0.32	0.26	0.45	0.21
2-decenal	0.20	0.13	0.23	0.28	0.19	0.07	0.17
β-terpineol	0.57	1.26	0.45	1.84	1.18	1.25	3.98
α-humulene	0.01	0.04	0.02	0.03	0.04	0.03	0.08
neral	0.01	0.05	0.01	0.06	0.04	0.09	0.15
borneol	0.06	0.10	0.10	0.11	0.08	0.06	0.07
α-terpinyl acetate	0.11	0.06	0.07	0.08	0.07	0.05	0.12
neryl acetate	0.04	0.06	0.03	0.05	0.06	0.07	0.04
citral	0.09	0.13	0.14	0.17	0.10	0.06	0.10
geranyl acetate	3.48	4.00	2.93	3.41	3.81	3.26	3.52
myrtenol	0.08	0.09	0.09	0.08	0.07	0.07	0.07
nerol	0.07	0.33	0.15	0.72	0.25	1.05	1.45
citronellol	2.49	1.47	2.64	2.27	1.28	0.89	0.60
geraniol	0.46	2.07	0.43	1.32	1.88	2.79	1.93
Fatty acids							
Palmitic acid	4.27	4.80	4.61	4.61	4.53	4.57	4.76
Stearic acid	2.35	2.90	2.75	2.49	2.47	2.01	2.47
Octadecenoic acid	0.20	0.41	0.38	0.57	0.33	0.16	2.13
Petroselenic acid	65.92	66.34	65.98	66.82	67.54	68.68	66.77
Oleic acid	9.76	8.82	8.94	9.00	8.75	8.32	7.64
Linoleic acid	15.75	14.50	15.08	14.20	14.22	14.21	13.74
Vaccenic acid	0.94	1.10	1.10	1.21	1.10	1.18	1.32
Other fatty acids	0.67	1.06	0.90	0.85	0.86	0.60	1.43

acids. Group 4 is characterized by the highest 2-decenal, borneol, and citral content, with second highest percentages for α-thujene, α-pinene, β-pinene, linalool oxide, and sabinene. In addition, this group presented the highest percentage for vaccenic acid other than ET1_Ic. Group 5 had highest values for β-pinene, myrcene, and α-humulene other than ET1_Ic, showing the second highest content for α-pinene other than RF2_IIIb, and for geranyl acetate. This group had the second highest proportion for petroselenic acid. As for the single populations, population RF2_IIIb had the highest percentage of α-pinene, β-pinene, myrtenal, octanol, and geraniol and the lowest content for camphor and many other volatile compounds. Notably, this population also had the highest level of petroselenic acid. Population ET1_Ic had the highest proportion of linalool oxide, β-terpineol, α-humulene, neral, α-terpinyl acetate, and nerol, with the lowest percentage of linalool, and the highest octadecenoic and vaccenic acid content (Table 11). These profiles document biochemical variation within the NCRPIS coriander collection, establishing clear differences among groups for essential-oil and fatty-acid composition, which may be valuable for specialized applications (López et al. in press).

Principal components analysis

From the combined PCA, there were eight principal components with eigenvalues above 1.0 that together explained more than 86% of the total variation in seed essential-oil and fatty-acid composition. The first two principal components had eigenvalues greater than 10, and, together with the third principal component, they explained more than 70% of the total variation. In relation to the eigenvector values, the primary volatile compounds contributed most heavily to the variation for the first two principal components, and palmitic, petroselenic, oleic, and linoleic acids contributed heavily to the variation for the third principal component. Although there was a trend for populations to cluster near the lowest absolute values for the three axes, there was a clear grouping pattern among them (Fig. 4), which corresponded more closely to the phenotypic subgroups to which they had been assigned. Populations from subgroup IIIb were still interspersed within other subgroups, but some patterning can be distinguished (Fig. 4). A notable highlight from Fig. 4 is that populations from phenotypic subgroups IIa and IIb clustered together at the extreme corner for lowest values for the first two principal components (especially population SY3_IIa), indicating that those populations are chemically very different from populations of the other subgroups, which is also supported by their distinctive morphology (i.e., tall plants with many, large basal leaves bearing many umbels with very small fruits).

PCA generally supported groups formed on the basis of the cluster analysis; similar results have been reported for other plant species (Cavaleiro et al. 2001; Mundina et al. 2001; Dunlop et al. 2003) where CA and PCA were complementary in elucidating

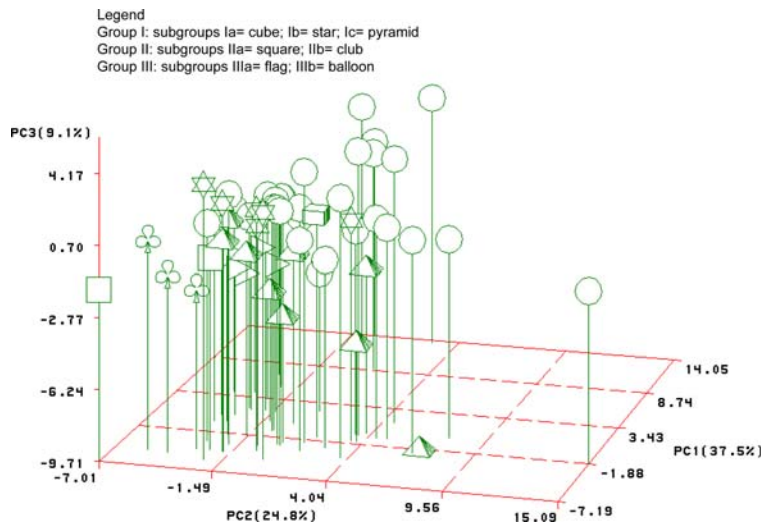

Fig. 4 Phenetic relationships among 60 coriander populations based on the first three principal components from biochemical data, presented as a three-dimensional scatter plot

relationships among populations in chemotaxonomic studies, with an additional advantage for PCA, in that it significantly reduces the number of original variables to a few principal components, which explain most of the total variation (Härdle and Simar 2003), although phenetic relationships are often better visualized on dendrograms from CA.

Correlations among phenotypic, biochemical, and genetic distances

There were no significant correlations between Euclidean distances from phenotypic data and modified Rogers' distances from AFLPs ($r = -0.003$, $P > 0.50$) or between biochemical and molecular distances ($r = 0.115$, $P > 0.07$). However, Euclidean distances from biochemical traits did correlate weakly with phenotypic distances ($r = 0.155$, $P = 0.05$). It is likely that human selection for specific plant phenotypes also modified seed chemistry. This is not surprising, since the use of its dried fruits as a spice was presumably the first intentional use for coriander (Diederichsen 1996). In contrast to our findings, significant correlations between molecular and biochemical data sets have been reported in other plant taxa (Adams 2000; Keskitalo et al. 2001; Vieira et al. 2001).

Molecular analysis

Cluster analysis

From the dendrogram based on AFLPs markers, we were able to define five clusters (Fig. 5), but there is

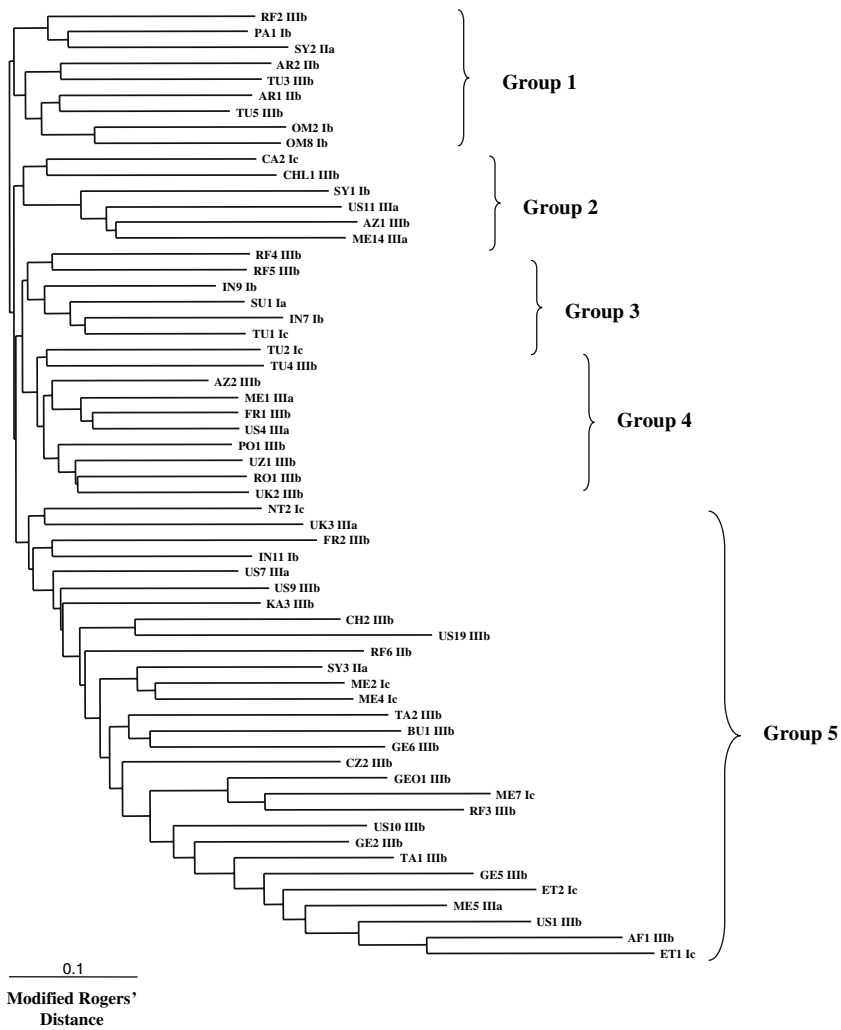

Fig. 5 Genetic relationships among 60 coriander populations based on modified Rogers' distances from 80 AFLP markers, presented as a dendrogram

no clear correspondence between our initial grouping based on phenotypic traits (Fig. 1), which somewhat supports the infraspecific classification proposed by Diederichsen and Hammer (2003), and one based on AFLP polymorphisms. More specifically, with regard to the two hypotheses posed earlier in relation to the geographically diverse, phenotypic subgroups Ic and IIIb, those two subgroups are randomly distributed among the five AFLP groups (χ^2, 4 d.f. = 2.17 and 2.29, respectively), suggestive of multiple evolutionary origins. However, there is evidence for close genetic relationships among pairs of phenotypically similar accessions from Mexico within subgroup Ic and from the Russian Federation within subgroup IIIb (as well as for a pair of subgroup Ib accessions from Oman).

Evidence for the widespread ''dispersal'' of types that are both phenotypically and genetically similar can be found in the PO1_IIIb, UZ1_IIIb, RO1_IIIb, UK2_IIIb cluster within AFLP Group 4, and in the CH2_IIIb, US19_IIIb pair and the TA2_IIIb, BU1_IIIb, GE6_IIIb cluster within AFLP Group 5.

Although the patterns presented in Fig. 5 suggest multiple evolutionary origins for similar phenotypes, it is important to note that there are many potential biological causes for incongruence between molecular and phenotypic datasets, including rapid morphological evolution based on relatively few loci, widespread hybridization events, and lineage sorting (reviewed by Wendel and Doyle 1998), as well as contributing technical issues. In the case of AFLP markers, the most important of these technical issues is the fact that co-migrating bands may not represent single, unique alleles (Vekemans et al. 2002), but this is most often true for the shortest fragments, which we removed from our analysis.

Analysis of molecular variance (AMOVA)

Table 12 presents the results of an AMOVA for the seven initial, phenotypic subgroups defined on the basis of the phenotypic distance matrix (Fig. 1). Variance components were significant for all the three sources of variation: among groups, among populations within groups, and within populations, supporting the proposed phenotypically based classification, but only very weakly, as reflected in the small value for the fixation index, F_{CT}. Less than 4% of the total variation in AFLP markers could be explained on the basis of the initial seven subgroups. Similar results (Table 13) were found for the biochemical groups (Fig. 3), although with a slightly higher percentage of the total variation (6%) attributed among groups, confirming the importance of intra-population variation in coriander populations.

As fixation indices (*Fs*) measure the amount of differentiation among subpopulations derived from the subdivision of an original population (Wright 1978), values for *Fs* range from 0 for non-differentiation to 1 for complete differentiation between an original population and its subpopulations, respectively. In this study, *Fs* values reflect similar levels of genetic differentiation among populations within groups and within populations, but the level of differentiation was markedly lower among groups (Tables 12, 13) than within groups or populations. We infer from the AMOVA results that international trade and an allogamous breeding system have contributed to genetically heterogeneous germplasm that has been and, likely continues to be, widely shared. Our findings differ only slightly from those for cultivated and wild carrot (*Daucus carota* L.), where no significant structure was detected by using

Table 12 AMOVA results for 80 loci (AFLP markers) and phenotypic variation

Source of variation	Sum of squares	Variance components[a]	Percentage variation
Among groups	197.38	0.44 (F_{CT} = 0.0365)	3.65***
Among populations within groups	1101.75	3.18 (F_{SC} = 0.272)	26.26 ***
Within populations	1368.08	8.49 (F_{ST} = 0.299)	70.08 ***
Total	2667.22	12.11	

From 60 populations, seven phenotypic subgroups were identified, based on Euclidean distances and the UPGMA clustering algorithm

[a] With corresponding fixation index in parentheses

*** Very highly significant (α = 0.001)

Table 13 AMOVA results for 80 loci (AFLP markers) and biochemical variation

Source of variation	Sum of squares	Variance components[a]	Percentage of variation
Among groups	246.039	0.74 (F_{CT} = 0.061)	6.10 ***
Among populations within groups	1035.470	2.97 (F_{SC} = 0.260)	24.30 ***
Within populations	1338.250	2.97 (F_{ST} = 0.305)	69.49 ***
Total	2619.759	12.15	

From 60 populations, seven biochemical groups were identified, based on Euclidean distances and the UPGMA clustering algorithm

[a] With corresponding fixation index in parentheses

*** Very highly significant (α = 0.001)

AFLP and ISSR markers (Bradeen et al. 2002). Similar results for AMOVA have been reported in other allogamous plants (Tero et al. 2003; Fjellheim and Rognli 2005; Rouf Mian et al. 2005).

Specific cases analogous to our findings include a study by Inocencio et al. (2005), who detected little genetic differentiation in two subspecies of the genus *Capparis* on the basis of AFLP markers, even when those subspecies were morphologically distinct, and a study of ryegrass (*Lolium* spp.) by Roldán-Ruiz et al. (2000) where phenotypic differences between turf and fodder types were not supported by AFLPs analysis. In contrast, in an AMOVA analysis for two self-pollinated species of *Stylosanthes* Sw., Sawkins et al. (2001) reported more variation among groups than within groups. Similarly, Cardoso et al. (2000) mentioned inbreeding as a cause for low within-population variation in heart-of-palm (*Euterpene edulis* Mart.). It is clear that inbreeding leads to an increase in variance between lines and a decrease in variance within them (Falconer and MacKay 1996). These findings collectively suggest that coriander conforms more closely to expectations of allogamous species than to autogamous ones, supporting past studies of its breeding system (Purseglove et al. 1981; Sethi 1981; Wróblewska 1992; Diederichsen 1996).

Our analysis of AFLP markers suggests that the current formal, botanical infraspecific classification of coriander, based on phenotypic and biochemical traits, may not reflect the evolutionary history of the crop. Thus, the systematic treatment of coriander by Diederichsen and Hammer (2003) may more closely reflect a utilitarian classification than one with a clear phylogenetic basis. However, it is important to emphasize that a utilitarian grouping of coriander cultivars still has great value for users, such as farmers, agronomists, plant breeders, and traders, even if there is no clear molecular support for a botanical classification at the infraspecific level. Fortunately, the International Code of Nomenclature for Cultivated Plants (ICNCP) recognizes that there are non-mutually exclusive taxonomic units, with no formal taxonomic value in botany, but with importance in practical classification related to agriculture. Those units are named Groups, which are defined as ''a category denoting an assemblage of cultivars, individual plants, or assemblages of plants on the basis of defined similarity'' (Brickell et al. 2004).

Conclusions

In summary, we can conclude that while strong phenotypic and biochemical differentiation exists among coriander germplasm accessions, our phenotypic classification differs somewhat from the infraspecific classification of coriander made by Diederichsen and Hammer (2003). Perhaps more importantly, patterns of phenotypic and biochemical differentiations in coriander are not well-captured by a random (neutral-gene) molecular approach. On the contrary, differentiation may be controlled by relatively few loci coding for important phenotypic and biochemical traits. The effects of human selection, under various environmental conditions, are clearly observable, but selection may be operating upon relatively few genes for too short a time, limiting completion of the genetic assimilation process (Schlinchting 2004), or may be operating in similar ways on different, unrelated base populations. In such situations, considerable phenotypic divergence could result without major differentiation within the broader genetic background. Other causes of the lack of genetic differentiation may include the wide exchange of coriander fruit as a spice around the world and a lack of breeding barriers among populations,

maintaining a relatively common genetic background among populations.

Furthermore, although only limited support was generated by our biochemical data for an earlier phenotypic infraspecific classification, it should be possible to combine the results from these phenotypic, biochemical, and molecular characterizations and use them to refine the current botanical classification in order to develop a utilitarian classification for coriander populations, by using the ICNCP's Group concept (Brickell et al. 2004). We plan to direct future efforts to that endeavor.

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